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Iso- and Anteiso-Fatty Acids in Bacteria: Biosynthesis, Function, and Taxonomic Significance†

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INTRODUCTION

Fatty acids are one of the most important building blocks of cellular materials. In bacterial cells, fatty acids occur mainly in the cell membranes as the acyl constituents of phospholipids. Membrane fatty acids can be divided into two major families on the basis of their biosynthetic relationships. One is the straight-chain fatty acid family, which includes palmitic, stearic, hexadecenoic, octadecenoic, cyclopropanic, 10-methylhexadecanoic, and 2- or 3-hydroxyl fatty acids. These fatty acids occur most commonly in bacteria. They are synthesized from acetyl coenzyme A (acetyl-CoA) as the primer and malonyl-CoA as the chain extender, followed, in some cases, by a modification of the fatty acid products.

The other is the branched-chain fatty acid family, which includes iso-, anteiso-, and ω-alicyclic fatty acids with or without a substitution (unsaturation and hydroxylation). The occurrence of these fatty acids in bacteria is not nearly as common as that of the straight-chain fatty acid family, but is still very significant (9, 77, 94, 118, 166). These fatty acids are synthesized in certain bacteria from iso, anteiso, or cyclic primer and malonyl-CoA with or without a subsequent modification.

The clear difference between these two families of cell membranes exists in the mechanism that controls their fluidity. The fluidity of membranes composed of straight-chain fatty acids is adjusted to the proper level by the inclusion of monounsaturated fatty acids, whereas that of membranes with branched-chain fatty acids is controlled mainly by 12- and 13-methyltetradecanoic acids. Thus, bacteria with the straight-chain membrane system usually require unsaturated fatty acids for growth, but these fatty acids are nonessential for bacteria with the branched-chain membrane system.

The occurrence of branched-chain fatty acids as major constituents in bacteria was first reported for *Bacillus subtilis* (67, 130). The genus *Bacillus* includes bacteria with a wide variety of physiological and biochemical properties, such as psychrophiles, mesophiles, thermophiles, insect pathogens, animal pathogens, antibiotic producers, and industrial enzyme producers. This genus has been the most extensively studied with respect to branched-chain fatty acids (77).

My previous review on branched-chain fatty acids in

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bacteria in 1977 was limited to the genus *Bacillus* (77). In the following decade, a sufficient volume of information on other genera has been accumulated to warrant a review of all bacterial genera with branched-chain lipids.

Many fatty acids with internal methyl or methylene or maltimethyl substitutions have been found in lipids of bacteria. These fatty acids are minor fatty acids constituents in many species and are not considered in this review. The review focuses primarily on the iso and anteiso series of branched-chain fatty acids in bacteria.

BIOSYNTHESIS OF FATTY ACIDS

Fatty acids synthetases from a wide variety of sources are divided into two groups on the basis of their physical structure: a multifunctional enzyme complex (type I) and the so-called soluble system, composed of seven individual enzymes (type II) (11).

Type I includes fatty acid synthetases from animals and yeasts; animal fatty acid synthetase is represented by a_2, a multifunctional polypeptide with a molecular weight of 5×10^5, and has seven distinct enzyme domains on one peptide (a), whereas yeast fatty acid synthetase is expressed by $\alpha_6\beta_6$ with a molecular weight of 2.3×10^6, and these seven domains are carried on two peptides (α and β) (159). Fungus fatty acid synthetase is essentially identical to yeast fatty acid synthetase. Type II includes fatty acid synthetase from higher plants and bacteria. Branched-chain fatty acid synthetase in bacteria also belongs to this group.

Knowledge about the biosynthesis of straight-chain fatty acids has increased rapidly since the discovery of malonyl-CoA as the substrate for the C_2 subunits in the synthesis (158). By mid-1970, the synthetic reaction in many organisms had been well established. Interestingly, the overall reaction is strikingly uniform throughout the systems of animals, plants, and microorganisms, in that acetyl-CoA is used as the primer and its carbon chain is elongated by the repeated condensation of malonyl-CoA to the primer, yielding palmitic acid as the major product. Furthermore, palmitic acid synthetases from various sources all require an acyl carrier protein (ACP) if they are the soluble system or include an ACP domain in their molecule if they are the multifunctional system (3, 16, 157).

Studies on the elucidation of the biosynthesis of branched-chain fatty acids in bacteria were initiated at about the same time as those involving palmitic acid synthesis, but progress of the work on branched-chain fatty acid synthesis has been very slow (77). This is due mainly to a low activity of the enzymes involved in branched-chain fatty acid synthesis. For instance, the overall rate of branched-chain fatty acid synthesis in *B. subtilis*, a representative of branched-chain fatty acid synthetase, is only 1/50 of the combined rate of straight-chain saturated and monounsaturated fatty acid synthesis found in *Escherichia coli*, a representative of straight-chain fatty acid synthetase (82). This low activity of branched-chain fatty acid synthetase in cell-free systems has severely impeded efforts to establish a detailed synthetic mechanism by using the individually isolated enzymes involved. However, available evidence strongly supports the idea that branched-chain fatty acids in bacteria are synthesized by a mechanism very similar to that of straight-chain fatty acid synthesis in *E. coli*, including the involvement of ACP in the synthetic reactions. The only difference between the two systems appears to be the substrate specificity of acyl-CoA:ACP transacylase. Thus, once an ACP derivative of acyl intermediates is formed, its elongation reaction can be carried out by either system.

In this review, the term "straight-chain fatty acid synthetase" is used synonymously with fatty acid synthetase (or palmitic acid synthetase), which produces straight-chain fatty acids, mainly palmitic and stearic acids, from acetyl-CoA as the best primer among short-chain acyl-CoA esters and malonyl-CoA as the chain extender (C_2 subunits). Branched-chain fatty acid synthetase is an enzyme system capable of synthesizing branched long-chain fatty acids from branched short-chain acyl-CoA esters as primers and malonyl-CoA as the chain extender. With this enzyme system, acetyl-CoA is hardly used as a primer. When α-keto acids are used as the primer sources, the system is designated a branched-chain fatty acid-synthesizing system to differentiate it from the branched-chain fatty acid synthetase, in which branched short-chain acyl-CoA esters are the primers.

Branched-chain fatty acids in bacteria are synthesized from two types of primer sources. The first type includes branched-chain α-keto acids, which are related to valine, leucine, and isoleucine and are used by nearly all bacteria with branched-chain lipids (77). The second type includes branched short-chain carboxylic acids, which are exogenously supplied and are used by only a small proportion of bacteria, mainly those incapable of utilizing branched-chain α-keto acids as primer sources. Some ruminal bacteria and mutants of *B. subtilis*, which require branched short-chain carboxylic acids for growth, fall into this minor group.

There is one other type, composed of only species of bacteria whose cellular fatty acids are mostly ω-alicyclic fatty acids. In these particular bacteria, the alicyclic fatty acids are synthesized from the corresponding endogenous cyclic carboxylic acid as the primer by a mechanism similar to that used by branched short-chain carboxylic acid-requiring bacteria. Thus, these bacteria can synthesize branched-chain fatty acids when the appropriate branched short-chain primers are provided to the organisms (88).

Branched-Chain Fatty Acids

De novo synthesis of saturated fatty acids in bacteria is carried out by two different types of fatty acid synthetases: straight-chain fatty acid synthetase and branched-chain fatty acid synthetase. The former carries out the following overall reaction:

$$\text{Acetyl-CoA} + 7\ \text{malonyl-CoA} + 14\text{NADPH} + 14\text{H}^+ = \text{palmitic acid} + 7\text{CO}_2 + 14\text{NADP} + 6\text{H}_2\text{O} + 8\text{CoA}$$

Most bacteria have a soluble system. Thus, the above reaction is catalyzed by seven individual enzymes: acetyl-CoA:ACP transacylase, malonyl-CoA:ACP transacylase, β-keto-acyl:ACP synthetase, β-keto-acyl:ACP reductase, D-(−)-β-hydroxyl-acyl:ACP dehydrase, enoyl:ACP reductase, and palmitoyl thioesterase. Detailed information on palmitic acid synthetase is given in references 3, 7, 43, 133, 141, 157, and 159.

In the synthesis of branched-chain fatty acids, malonyl-CoA also functions as the chain extender. Thus, the mechanism of chain extension for the synthesis of branched-chain fatty acids is essentially the same as that for the synthesis of straight-chain fatty acids. The only difference between the two reactions lies in their respective primers and products.

Short-chain carboxylic acids as primer sources. Some species, as well as certain mutants of other species, require branched short-chain carboxylic acids, namely isobutyric, isovaleric, and 2-methylbutryic acids, for growth. These

TABLE 1. Kinetic constants for the two decarboxylases[a]

Substrate	BCKA decarboxylase[b]		Pyruvate decarboxylase	
	K_m (μM)	V_{max} (nmol/min/mg)	K_m (μM)	V_{max} (nmol/min/mg)
L-α-Keto-β-methylvalerate	<1	17.8	58.2	16.7
α-Ketoisovalerate	<1	13.3	33.6	53.0
α-Ketoisocaproate	<1	5.6	88.8	11.8
Pyruvate	51.1	15.2	10.2	60.4

[a] Reproduced from reference 117 with permission.
[b] BCKA, Branched-chain α-keto acid.

organisms include certain ruminal bacteria and mutants of *B. subtilis* (14, 90, 164). In these cases, short-chain carboxylic acids added to the culture media are transformed to their related CoA esters. They are then used as primers, to yield branched long-chain fatty acids, according to the following equation:

$$\text{Isobutyryl-CoA} + 6\ \text{malonyl-CoA} + 12\text{NADPH} + 12\text{H}^+ = \text{isopalmitic acid} + 6\text{CO}_2 + 12\text{NADP} + 5\text{H}_2\text{O} + 7\text{CoA}$$

Branched-chain fatty acid synthetase has a primer preference for acyl-CoA esters with three to six carbons. Thus, when various branched short-chain carboxylic acids with six carbons are added to a culture medium, *B. subtilis* uses them as primer sources to synthesize the related long-chain fatty acids, including those which occur in nature and those which do not (73). Acetyl-CoA is a poor primer, having only a small percentage of the activity of butyryl-CoA (82). The synthesis of straight-chain fatty acids in bacteria possessing branched-chain fatty acid synthetase will be considered below.

Some eukaryotic straight-chain fatty acid synthetases, however, accept short-chain acyl-CoA esters with three to six carbons as primers. Fatty acid synthetase from adipose tissues utilizes branched short-chain acyl-CoA esters as excellent primers to synthesize the related branched long-chain fatty acids (56). Thus, if the proper donating system for branched short-chain acyl-CoA esters is provided, these tissues would be capable of synthesizing branched long-chain fatty acids and would consequently have branched-chain lipid membranes. Mammary gland fatty acid synthetase, although capable of utilizing short-chain acyl-CoA esters, still falls in the category of straight-chain fatty acid synthetase, since butyryl-CoA is nearly as active as acetyl-CoA as a primer (86).

Acyl-CoA esters added as primers are initially converted to their ACP derivatives by the following reaction before condensation takes place:

$$\text{Acyl-CoA} + \text{ACP} \rightleftharpoons \text{acyl-ACP} + \text{CoA}$$

The difference between branched-chain fatty acid synthetase and straight-chain fatty acid synthetase is due to the substrate specificity of the enzyme (acyl-CoA:ACP transacylase) catalyzing the above reaction. Branched-chain fatty acid synthetase cannot efficiently convert acetyl-CoA to acetyl-ACP. If, however, acetyl-acyl carrier protein is provided to the synthetase, it can synthesize straight-chain fatty acids as well as branched-chain fatty acids from branched short-chain acyl-CoA esters, such as isobutyryl-CoA (15, 84). The converse is true for straight-chain fatty acid synthetase. Straight-chain fatty acid synthetase from *E. coli* cannot effectively use isobutyryl-CoA as a primer. If isobutyryl-ACP derivative is provided to the *E. coli* synthetase, it produces the related branched-chain fatty acids.

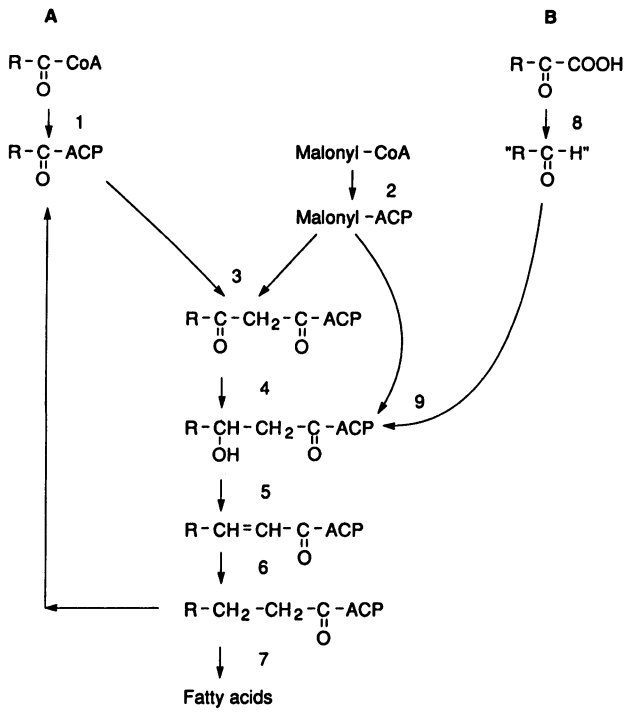

FIG. 1. Pathways of branched-chain fatty acid synthesis in *B. subtilis* and other organisms, which possess branched-chain fatty acids as major cellular fatty acids. A, Pathway of the synthesis from branched-chain acyl-CoA ester as a primer; B, the other pathway of the synthesis from branched-chain α-keto acid as a primer source.

α-Keto acids as primer sources. Branched-chain fatty acids in bacteria are synthesized mainly by using α-keto acids as their primer source. A branched-chain fatty acid-synthesizing system from *B. subtilis* shows that short-chain carboxylic acid-CoA esters are not main intermediates in the incorporation of branched-chain α-keto acids into the related branched long-chain fatty acids. The synthesis of acyl-CoA esters from branched-chain α-keto acids by branched-chain α-keto acid dehydrogenase, which is present in *B. subtilis*, requires CoA and NAD as cofactors (114). These cofactors, however, have been shown to be not required for the synthesis of fatty acids from α-keto acid substrates; rather, they inhibit the synthesis (76). This indicates that a decarboxylase, but not a dehydrogenase, is involved in the synthesis of the primer. Recently, two α-keto acid decarboxylases were isolated from cell extracts of *B. subtilis*. Immunoprecipitation experiments revealed that the branched-chain α-keto acid decarboxylase, but not the other decarboxylase (pyruvate decarboxylase), is essential to the incorporation of branched-chain α-keto acid substrates into fatty acids (117). Both α-keto acid decarboxylases have a similar range of substrate specificity, but branched-chain α-keto acid decarboxylase has a much higher affinity for branched-chain substrates than pyruvic acid decarboxylase does (Table 1). The concentration of branched-chain α-keto acids in the metabolic pool of bacteria is expected to be low. However, branched-chain α-keto acid decarboxylase, which has a high affinity toward the α-keto acids, would still be functional in cells.

Figure 1 shows the pathway proposed for the synthesis of branched-chain fatty acids in *B. subtilis*. Column A, combined with the central column, shows the synthesis of

branched-chain fatty acids from branched short-chain carboxylic acid-CoA esters. As mentioned above, the substrate specificity of the enzyme catalyzing reaction 1 is crucial to differentiating branched-chain fatty acid synthetase from palmitic acid synthetase.

Column B, along with the central column, represents the synthesis of branched-chain fatty acids from branched-chain α-keto acids. Reaction 8 is catalyzed by branched-chain α-keto acid decarboxylase. Studies to identify the enzyme carrying out the condensation reaction, reaction 9, are being conducted in this laboratory. The primer is assumed to be an aldehyde thiaminepyrophosphate derivative or an aldehyde-enzyme complex, which condenses with a malonyl-ACP derivative. Once the β-hydroxyacyl-ACP derivative is formed, the remaining reactions are carried out by the same process as in the *E. coli* system.

Recently, another condensing enzyme has been found in *E. coli*. In addition to the known condensing enzyme, which uses an acetyl-ACP derivative as the primer, the organism possesses a newly discovered condensing enzyme, which uses acetyl-CoA, but not its ACP. It is shown to be insensitive to the antibiotic cerulenin, whereas the known enzyme is sensitive (61, 62). This suggests that *B. subtilis* may possess two condensing enzymes in a way similar to *E. coli*. Thus, reactions 3 and 9 in Fig. 1 may be catalyzed by two different enzymes.

ω-Alicyclic Fatty Acids

Studies have shown that ω-alicyclic fatty acids, either cyclohexyl or cycloheptyl, are the major membrane fatty acids in several species of bacteria (28, 31, 125). Branched-chain fatty acids are also present in these bacteria. The fatty acid synthetase of these bacteria is considered to be catalytically identical to that of bacteria which produce branched-chain fatty acids as the major components of their cellular fatty acids. The only difference is that in these bacteria the supply of cyclic carboxylic acid-CoA esters is much greater than that of branched-chain primers. In support of this idea, fatty acid synthetase of *Curtobacterium pusillum*, an organism containing 80% ω-cyclohexyl fatty acids, uses branched short-chain acyl-CoA esters as well as cyclohexyl carboxylic acid CoA ester as primers, but does not use acetyl-CoA (84). ω-Cycloheptanyl fatty acids, which occur in *Bacillus cycloheptanicus*, are likely to be produced by a similar mechanism (32).

The synthesis of cyclic primers in these bacteria is not well understood. Cyclohexylcarboxylic acid-CoA ester seems to be produced from shikimic acid, but the detailed synthetic pathway is unknown (119).

Straight-Chain Fatty Acids

Straight-chain fatty acids—mainly myristic, palmitic, and stearic acids—occur usually as minor fatty acids in a range up to 10% of the total cellular fatty acids in bacteria which contain mostly branched-chain fatty acids. Acetyl-CoA, unlike isobutyryl-CoA, is not a good primer for fatty acid synthetase from these bacteria (82), but it should be able to serve as the primer to yield a small amount of straight-chain fatty acids present in the organisms. Some organisms in this group, however, possess an unusually high proportion of straight-chain fatty acids. For instance, *Bacillus insolitus* possesses 36% straight-chain fatty acids (83). This anomaly raises the question of the nature of the primer for palmitic acid synthesis in such bacteria.

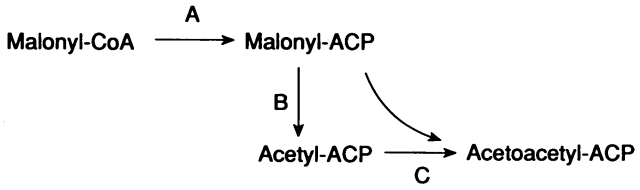

FIG. 2. Pathway proposed for straight-chain fatty acid synthesis in *B. subtilis* and other organisms, which possess branched-chain fatty acids as major cellular fatty acids. A, B, and C indicate enzymatic steps.

It is known that acetyl-ACP derivative, but not acetyl-CoA, serves as an excellent primer for fatty acid synthetase from *B. subtilis* (15). The following reaction is proposed for the synthesis of acetyl-ACP (83):

$$\text{Malonyl-ACP} \rightleftharpoons \text{acetyl-ACP} + CO_2$$

The condensing enzyme from *E. coli* is capable of catalyzing this reaction (2, 48). Thus, the enzyme may provide acetyl-ACP for the synthesis of straight-chain fatty acids in bacteria with the branched-chain lipid system. This is consistent with the observation that *B. insolitus*, which possesses a high activity of branched-chain fatty acid synthetase (thus condensing enzyme) contains a large proportion of straight-chain fatty acids (83). Therefore, a malonyl-acyl carrier protein derivative may serve both functions, as the primer and the chain extender.

Figure 2 shows a pathway proposed for the biosynthesis of straight-chain fatty acids in bacteria, whose fatty acids are mostly branched-chain fatty acids. Reaction A is catalyzed by malonyl-CoA:ACP transacylase. Reactions B and C are both catalyzed by a single enzyme, condensing enzyme (β-ketoacyl-ACP synthetase). Once acetoacetyl-ACP is produced, it is transformed by a series of enzyme reactions, which are well established in synthetase of *E. coli*, yeasts, plants, and animals, to yield palmitic acid. In support of the proposed pathway, the ACP-dependent decarboxylation (reactions B) and the ACP-dependent formation of a condensation product (reaction C) from malonyl-CoA has been detected in a partially purified preparation of *B. subtilis* cell-free extracts (66a).

STEREOSPECIFICITY

Stereospecificity is a fundamental property of biological reactions catalyzed by enzymes. Thus one, but not both, of the enantiomers of asymmetric compounds occurs predominantly in living organisms.

12-Methyltetradecanoic (anteiso-C_{15}) and 14-methylhexadecanoic (anteiso-C_{17}) acids occur in many bacteria as major constituents of membrane lipids. The stereospecificity in the biosynthesis of these anteiso-fatty acids has been discussed previously (77). The *S* isomers of 2-methylbutyric acid and 2-keto-3-methylvaleric acid are natural precursors in anteiso-fatty acid synthesis in *B. subtilis*.

In support of this, branched-chain α-keto acid decarboxylase, which is essential for the incorporation of α-keto acid into fatty acids in *B. subtilis*, has been shown to be specific toward the *S* isomer of 2-keto-3-methylvaleric acid (117). Interestingly, the same stereospecificity has been observed with pyruvic acid decarboxylase (117) and branched-chain α-keto acid dehydrogenase (114), although those are not involved in the fatty acid synthesis.

TABLE 2. Identification of cellular fatty acids of ice-nucleating isolate W-11[a]

Peak no.	Fatty acid			Equivalent chain length on column:		
	Chemical name	Abbreviation	% of culture[b]	DX-4 (polar)	SP-2100 (nonpolar)	ΔECL[c]
1	Lauric	$C_{12:0}$	4.0	11.98	12.00	−0.02
2	Myristic	$C_{14:0}$	0.4	13.96	14.00	−0.04
3	3-Hydroxydecanoic	$C_{10:0}$ (3-OH)	1.4	14.18	11.38	2.80
4	2-Hydroxydodecanoic	$C_{12:0}$ (2-OH)	0.9	15.30	13.16	2.14
5	Palmitic	$C_{16:0}$	34.5	16.01	16.00	0.01
6	*cis*-9-Hexadecenoic	$C_{16:1}$ (*cis*)	40.3	16.10	15.75	0.35
7	3-Hydroxydodecanoic	$C_{12:0}$ (3-OH)	0.6	16.16	13.50	2.66
8	Not defined	$C_{17:0}$ (Cy)	2.4	17.10	16.80	0.33
9	Stearic	$C_{18:0}$	0.8	18.00	18.02	−0.02
10	*cis*-11-Octadecenoic	$C_{18:1}$ (*cis*)	15.3	18.09	17.71	0.38

[a] Reproduced from reference 80 with permission.
[b] Composition of culture grown at 30°C on Trypticase soy broth.
[c] Difference in equivalent lengths of fatty acids measured on polar and nonpolar columns.

B. subtilis selectively uses the *S* isomer from racemic 2-methylbutyric acid or racemic 2-keto-3-methylvaleric acid, which are provided to the culture medium, to yield *S*-anteiso-fatty acids (69). Essentially identical results were obtained in the synthesis of cyclopentenyl fatty acids from 2-cyclopentenyl carboxylic acid in the same organisms (78). Interestingly, these bacterial cyclopentenyl fatty acids have the *S* configuration, opposite to that of the plant acids (100).

This stringent stereospecificity in the anteiso-fatty acid synthesis, however, is not observed in two *B. subtilis* mutants, which require 2-methylbutyric acid. The organisms use either of the isomers of 2-methylbutyric acid to synthesize the related anteiso-fatty acids, although the *S* isomer is more effective than the *R* isomer for growth. When racemic 2-methylbutyric acid is provided, the organisms synthesize racemic fatty acids, rich in *S*-anteiso-fatty acids (an optical purity of approximately 50%) (81). This indicates that the unnatural isomer of acyl constituents in cell membranes can fulfil functions as well as the natural isomer to support growth.

The detailed mechanism for this lack of stereospecificity in anteiso-fatty acid synthesis in *B. subtilis* mutants remains to be discovered.

CELLULAR BRANCHED-CHAIN FATTY ACIDS IN BACTERIAL SYSTEMATICS

Fatty Acid Analysis

These days, fatty acids in bacterial lipids are routinely analyzed by gas-liquid chromatography. In addition, other methods such as nuclear magnetic resonance spectroscopy, infrared spectroscopy, mass spectrometry, and thin-layer chromatography have been used to aid the gas-liquid chromatographic identification of fatty acids.

Since the late 1950s there have been major advances in the gas-liquid chromatographic technique, in particular in column technology, from packed columns, support-coated open-tubular columns, and wall-coated capillary columns to the chemically bonded capillary columns used today. There is no problem in identifying bacterial straight-chain fatty acids, with or without unsaturation, by gas-liquid chromatography. Fatty acid peaks of a common bacterial lipid sample, such as *n*-$C_{14:0}$, *n*-$C_{16:0}$, *n*-$C_{16:1}$, *n*-$C_{18:0}$, and *n*-$C_{18:1}$ on the chromatogram, are well resolved by using a short (2-m) column. The analysis of branched-chain fatty acids, however, requires much more stringent conditions. For example, the resolution of a pair of iso-C_{15} and anteiso-C_{15} acids commonly occurring in certain bacteria requires a column with at least a theoretical plate of 16,000. An 8-m pack column or 10-m capillary column is needed for this achievement.

Gas-liquid chromatography is used to identify fatty acids on the basis of their retention characteristics. A few different fatty acids in a given sample are likely to have the same retention time. For this reason, the class separation of fatty acids prior to gas-liquid chromatography makes identification easier and more reliable.

The recommended protocol for fatty acids analysis is as follows. (i) Fatty acids isolated from lipids of a bacterium are converted to methyl esters. (ii) A sample of the methyl esters is then fractionated to saturated esters, monounsaturated esters, diunsaturated esters, and hydroxy esters, by algentine thin-layer chromatography. (iii) The equivalent chain length of fatty acid methyl esters of four fractions is determined by gas-liquid chromatography on two capillary columns, a polar and a nonpolar column, and is compared with that of the authentic samples to identify the fatty acid methyl esters. (iv) The location of the methyl side chain, desaturation, and hydroxy group along the carbon chain of fatty acids is determined by the combined system of gas-liquid chromatography and mass spectrometry. (v) The geometric isomers of unsaturated fatty acids are determined by infrared spectroscopy.

In particular, the equivalent chain lengths of a fatty acid methyl ester measured on two columns are very useful in identifying the fatty acid. Table 2 provides a good example of this approach. The difference between two equivalent chain lengths is zero for saturated fatty acids, 0.35 to 0.38 for monounsaturated fatty acids, 2.14 for 2-hydroxyl fatty acids, and 2.66 to 2.80 for 3-hydroxyl fatty acids. This tentative identification is confirmed by the combined system of gas chromatography and mass spectrometry (80).

Two general rules should be considered. The first is that pairs of saturated fatty acids with a difference of two carbons, such as iso-C_{15} and iso-C_{17}, and anteiso-C_{15} and anteiso-C_{17}, always occur in lipids of a bacterium, although in some cases the one may be much more abundant than the other in a pair. This is due to their biosynthetic mechanism. The second is that the presence of even-numbered anteiso-fatty acids has been occasionally reported in the literature, based only on gas-liquid chromatographic evidence. In na-

ture, the occurrence of the anteiso-C_6 primer is extremely rare, and sufficient evidence should be provided before making the identification of even-numbered anteiso-fatty acids. Unsaturated fatty acids have been mistakenly identified as even-numbered anteiso-fatty acids.

Branched-Chain Fatty Acids in Bacterial Species

The fatty acid composition of bacteria has been used as an aid in their identification. However, only a limited number of bacteria have unusual fatty acids (such as tuberculostearic, lactobacillic, and mycolic acids) which are unique to the respective specific species of bacteria and can be used to identify them (4). The majority of bacteria have a simple fatty acid composition, comprising straight-chain fatty acids (for instance, a mixture of myristic, palmitic, stearic, and *cis*-9-hexadecenoic acids). Unfortunately, this simple pattern does not provide enough variety for distinguishing bacteria.

By contrast, branched-chain fatty acids of the iso and anteiso series occur widely in bacteria, give a complex pattern, and are therefore of much greater value in improving bacterial systematics.

It should be noted that the fatty acid profile of a bacterium with a branched-chain lipid type is affected by its growth conditions, e.g., growth phase, temperature, pH, oxygen supply, carbon sources, and an excessive supply of single primer sources (isoleucine, leucine, valine, and related substrates). In my laboratory a consistent fatty acid profile characteristic of such organisms has been obtained by growth in a common complex liquid medium. The pH, temperature, and oxygen level should not necessarily be those that give the best growth rate, but should not cause any stress response in the organisms. The cultures are harvested in the early stationary phase and used for fatty acid analysis.

Table 3 shows species of bacteria which possess branched-chain fatty acids in excess of 20% of the total cellular fatty acids. In some cases, species below this limit are included for special purposes. The lower limit was chosen because it represents the amount of branched-chain fatty acids essential to the growth of a mutant of *B. subtilis* (81). Ideally, the table should list only bacterial species which have branched-chain fatty acid synthetase. This can be determined by measuring the ratio of activity of butyryl-CoA to that of acetyl-CoA in their incorporation into fatty acids by fatty acid synthetase (82). This ratio is 100:1 to 3 for branched-chain fatty acid synthetase, and 1:10 for straight-chain fatty acid synthetase. At present, however, the ratio is determined with only a limited number of bacterial species.

Most species in which the branched-chain fatty acids represent less than 20% of the total fatty acids are likely to possess straight-chain fatty acid synthetase. A large amount of branched-chain primer sources, such as isobutyric, isovaleric, and 2-methylbutyric acids, supplied exogenously or endogenously to straight-chain fatty acid synthetase would cause it to synthesize branched-chain fatty acids. This is the case for some of the ruminal bacteria (109) and *Acholeplasma laidlawii* B (131).

Table 3 lists species of bacteria belonging to 56 genera out of a total of 398 genera, in the order in which they appear in *Bergey's Manual of Systematic Bacteriology*. This manual consists of four volumes, published in 1984 (87), 1986 (138), 1989 (139), and 1989 (165). The genera are divided into four groups: gram-negative (volume 1), gram-positive (volume 2), prosthecate and gliding (volume 3), and actinomyces (volume 4) bacteria. The group of gram-positive bacteria contains the largest number of genera, the gram-negative bacteria the next largest, the actinomyces bacteria the third largest, and the prosthecate and gliding bacteria the fewest.

The genera which are homogeneous among species with respect to the possession of branched-chain fatty acids include *Legionella*, *Flavobacterium*, and *Bacteroides* in the gram-negative bacteria; *Staphylococcus*, *Bacillus*, and *Arthrobacter* in the gram-positive bacteria; *Cytophaga* and *Myxococcus* in the prosthecate and gliding bacteria; and *Streptomyces* in the actinomyces bacteria. Thus, the validity of new species to be classified in these genera can be examined on the basis of the major occurrence of branched-chain fatty acids in lipids of the organisms.

The genera *Micrococcus*, *Clostridium*, and *Corynebacterium* are heterogeneous; i.e., they include species that possess straight-chain fatty acids alone and those that possess branched-chain fatty acids. Most *Clostridium* species do not have branched-chain fatty acids, with the exception of four species listed in Table 3. *Corynebacterium* species, however, have equal mixtures of the two types. The occurrence of high proportions of iso- and anteiso-fatty acids in *Corynebacterium diphtheriae* reported earlier (10) has not been substantiated. All of 74 strains of mycolic acid-containing coryneform bacteria, including *C. diphtheriae*, possess straight-chain fatty acids. Iso- and anteiso-acids are not present (23). Two *Micrococcus* species, *Micrococcus radiodurans* and *M. radiophilus*, which possess only straight-chain fatty acids, are now placed under the genus *Deinococcus* (13). This indicates the usefulness of fatty acid patterns in bacterial systematics.

Components Affecting Fatty Acid Patterns

Cellular fatty acid patterns of bacteria can be grouped into several types. For *Bacillus* species, four factors which contribute to fatty acid patterns have been identified on the basis of biochemical mechanisms. These factors are the ratio of three classes of branched-chain fatty acids, the proportion of straight-chain fatty acids, the occurrence of unsaturated fatty acids, and the relatively high proportion of unique fatty acids (77). An additional factor, namely the occurrence of hydroxy fatty acids, must be considered in examining fatty acid patterns of whole bacteria having branched-chain fatty acids as major cellular fatty acids. These five factors will be considered in relation to fatty acid patterns in the following sections.

Ratio of three classes of branched-chain fatty acids. Many species of *Bacillus* and other genera listed in Table 3 have branched-chain fatty acids as major cellular fatty acids. The gas-liquid chromatogram of methyl esters of the total fatty acids from one of the species usually shows the iso-$C_{15:0}$ or anteiso-$C_{15:0}$ peak as the highest among the fatty acid peaks. On this basis, species in some genera, such as *Bacillus* (70, 71), *Bacteroides* (103), and *Propionibacterium* (111), are divided into two groups. In some cases, the two peaks become nearly equal in intensity, such as in *Bacillus macerans* (70). The peak of iso-C_{16} acid seldom becomes the largest one. Such rare cases are *Legionella pneumophila* (38, 102) and *Bacillus naganoensis* (145).

The largest component of the cellular fatty acids of a bacterium with a branched-chain lipid type is related to the relative size of metabolic pools of α-ketoisocaproate, α-keto-β-methylvalerate, and α-ketoisovalerate. These pools are the sources of primers in the synthesis of iso-odd, anteiso-odd, and iso-even fatty acids, respectively, in cells (68). The

TABLE 3. Fatty acid composition

Bacterium (% of fatty acids that are branched)	Reference
Gram-negative bacteria	
Spirochetes	
Spirochaeta: *S. aurantia* (51%), *S. litoralis* (68%), *S. stenostrepta* (66%), *S. zuelzerae* (47%)	66, 97, 108
Aerobic Rods and Cocci	
Pseudomonas: *P.* (*Xanthomonas*)[a] *maltophilia* (47%), *P.* (*Alteromonas*)[a] *putrefaciens* (48%), *P. rubescens* (33%)[b]	60, 112
Xanthomonas: *X. campestris* (58%), *X. citri*[b] (51%)	59
Legionella: *L. anisa* (75%), *L. bozemanii* (68%), *L. cherrii* (51%), *L. dumoffii* (63%), *L. erythra* (33%), *L. feelei* (37%), *L. gormanii* (56%), *L. hackeliae* (62%), *L. jamestoniensis* (78%), *L. jordanis* (84%), *L. longbeachae* (44%), *L. maceachernii* (71%), *L. micdadei* (71%), *L. oakridgensis* (32%), *L. parisiensis* (55%), *L. pneumophila* (64%), *L. rubrilucens* (46%), *L. sainthelensi* (57%), *L. santicrucis* (38%), *L. spiritensis* (52%), *L. steigerwaltii* (36%), *L. wadsworthii* (73%)	102
Moraxella (*Branhamella*)[a]: *M. catarrhalis* (21%)	54
Thermus: *T. aquaticus* (94%), *T. flavus* (98%) *T. ruber* (62%)	63, 98, 120
Flavobacterium: *F. aquatile* (56%), *F. balustinum* (97%), *F. breve* (58%), *F. ferrugineum* (48%), *F. flavescens*[b] (74%), *F. fuscum*[b] (80%), *F. halmephilium* (43%), *F. heparinum* (73%), *F. lutescens* (75%), *F. meningosepticum* (85%), *F. odoratum* (95%), *F. sewanense* (60%), *F. sulfureum* subsp. *miyamizu* (80%), *F. thalpophilum* (31%), *F. thermophilum* (99%)	30, 110, 120, 121, 148
Anaerobic Straight, Curved, and Helical Rods	
Bacteroides: *B. asaccharolyticus* (96%), *B. bivius* (major), *B. buccalis* (66%), *B. disiens* (major), *B. distasonis* (major), *B. fragilis* (79%), *B. gingivalis* (major), *B. intermedius* (88%), *B. macacae* (major), *B. melaninogenicus* subsp. *levii* (88%), *B. melaninogenicus* subsp. *melaninogenicus* (92%), *B. oralis* (94%), *B. oulorum* (76%), *B. pentosaceus* (70%), *B. putredinis* (62%), *B. thetaiotaomicron* (78%), *B. vulgatus* (55%)	12, 41, 92, 103, 104, 134
Succinimonas: *S. amylolytica* (70%)	57
Dissimilatory Sulfate or Sulfur Reducers	
Desulfovibrio: *D. africanus* (30%), *D. desulfuricans* (61%), *D. gigas* (57%), *D. salexigens* (55%), *D. vulgaris* (53%)	99, 151
Gram-positive bacteria	
Cocci	
Micrococcus: M. agilis (81%), *M. conglomeratus*[b] (99%), *M. halobius* (99%), *M. luteus* (87%), *M. mucilaginosus* (73%), *M. roseus* (11%, 78%), *M. tetragenus* (100%), *M. varians* (80%)	44, 64
Stomatococcus: *S. mucilaginosus* (75%)	64
Planococcus: *P. citreus* (80%), *P. kocuril* (73%)	101
Marinococcus: *M. albus*[b] (95%), *M.* (*Planococcus*)[a] *halophilus* (92%)	51
Staphylococcus: *S. aureus* (81%), *S. capitis* (49%), *S. cohnii* (84%), *S. epidermidis* (81%), *S. haemolyticus* (84%), *S. hominis* (72%), *S. hyicus* (73%), *S. intermedius* (85%), *S. lentus* (79%), *S. saprophyticus* (73%), *S. sciuri* (94%), *S. simulans* (67%), *S. warneri* (37%), *S. xylosus* (55%)	115
Streptococcus: *S. agalactiae* (41%), *S. equi* (39%), *S. faecalis* (21%), *S. pyogenes* (55%), *S. salivarius* (35%)	37
Peptostreptococcus: *P. anaerobius* (62%)	93
Ruminococcus: *R. albus* (50%), *R. flavefaciens* (75%)	57
Sarcina: *S. lutea* (100%), *S. maxima* (18%), *S. tetragenus* (100%), *S. ventriculi* (20%)	163
Endosporeforming Rods and Cocci	
Bacillus: *B. acidocaldarius* (36%, 59%[c]) *B. acidoterrestris* (major[c]), *B. alcalophilus* (92%), *B. alvei* (90%), *B. anthracis* (83%), *B. brevis* (84%), *B. caldolyticus* (72%), *B. caldotenax* (78%), *B. cereus* (80%), *B. circulans* (74%), *B. coagulans* (89%), *B. cycloheptanicus* (90%[d]), *B. firmus* (90%), *B. globisporus* (87%), *B. insolitus* (63%), *B. inulinus* (94%), *B. laevolacticus* (97%), *B. larae* (78%), *B. lentimorbus* (56%), *B. licheniformis* (91%), *B. macerans* (89%), *B. megaterium* (89%), *B. mixolactis* (94%), *B. mycoides* (75%), *B. naganoensis* (99%), *B. natto* (88%), *B. polymyxa* (70%), *B. popilliae* (74%), *B. psychrophilus* (93%), *B. pumilus* (73%), *B. racemilacticus* (96%), *B. stearothermophilus* (44%), *B. subtilis* (95%), *B. thuringiensis* (81%)	18, 32, 68, 71, 77, 83, 145, 150, 161
Sporolactobacillus: *S. inulinus* (95%)	150
Clostridium: *C. difficile* (21%), *C. sordellii* (29%), *C. tartarivorum* (48%), *C. thermocellum* (75%), *C. thermosaccharolyticum* (55%)	17, 39, 52
Sporosarcina: *S. ureae* (73%)	163
Desulfotomaculum: *D. nigrificans* (33%)	151
Regular Nonsporeforming Rods	
Listeria: *L. denitrificans* (79%), *L. grayi* (65%), *L. monocytogenes* (56%)	25, 127
Brochothrix: *B. thermosphacta* (*Microbacterium thermosphactum*)[a] (72%)	135
Renibacterium: *R. salmoninarum* (99%)	19
Kurthia: *K. zopfii* (78%)	46
Irregular Nonsporeforming Rods	
Corynebacterium: *C. aquaticum* (99%), *C. cyclohexanicum*[b] (95%), *C.* (*Rhodococcus*)[a] *equi* (35%), *C.* (*Curtobacterium*)[a] *flaccumfaciens* subsp. *betae* (94%), *C. insidiosum* (84%), *C. iranicum* (94%), *C. manihot* (93%), *C. mediolanum* (99%), *C. michiganense* (74%), *C. michiganense* subsp. *michiganense* (93%), *C. nebraskense* (98%), *C. ovis* (89% in phospholipids), *C.* (*Curtobacterium*)[a] *poinsettiae* (93%), *C. pseudodiphtheriticum* (33%), *C. sepedonicum* (78%), *C. tritici* (96%)	8, 21, 24, 50, 79, 144

Continued on following page

TABLE 3—*Continued*

Bacterium (% of fatty acids that are branched)	Reference
Arthrobacter: *A. radiotolerans* (12%, 71%[e]), *A. atrocyaneus* (96%), *A. aurescens* (94%), *A. citreus* (94%), *A. globiformis* (85%), *A. nicotianae* (93%), *A. oxydans* (74%), *A. pascens* (59%), *A. ramosus* (91%), *A. (Pimelobacter)*[a] *simplex* (43%), *A. (Pimelobacter)*[a] *tumescens* (4%), *A. ureafaciens* (94%)	8, 142
Brevibacterium: *B. fermentans* (97%), *B. iodium* (98%), *B. linens* (98%), *B. saperde* (90%), *B. testaceum* (95%)	21, 23, 26
Curtobacterium: *C. citreum* (98%), *C. luteum* (99%), *C. pusillum* (10%, 90%[c])	22, 143
Cellulomonas: *C. biazotea* (82%), *C. flavigena* (54%), *C. subalbus* (70%)	21
Agromyces: *A. ramosus* (99%)	20
Rothia: *R. dentocariosa* (48%)	123
Propionibacterium: *P. acidipropionici (arabinosum)*[a] (50%), *P. acidipropionici (pentosaceum)*[a] (42%), *P. acnes* (57%), *P. acnes (anaerobium)*[a] (78%), *P. acnes (diphtheroides)*[a] (60%), *P. acnes (liquifaciens)*[a] (66%), *P. freudenreichii* (63%), *P. freudenreichii (shermanii)*[a] (59%), *P. granulosum* (50%), *P. jensenii* (57%), *P. jensenii (zeae)*[a] (56%), *P. thoenii* (52%)	111
Eubacterium: *E. lentum* (65%)	155
Prosthecate gliding bacteria	
Nonfruiting Gliding	
Cytophaga: *C. aquatilis* (49%), *C. arvensicola* (54%), *C. flevensis* (37%), *C. hutchinsonii* (45%), *C. johnsonae* (50%)	121, 122, 168, 169
Sphingobacterium: *S. mizutae*[b](30%), *S. (Flavobacterium)*[a] *multivorum*[b] (22%)	168
Capnocytophaga: *C. gingivalis* (82%), *C. ochracea* (80%), *C. sputigena* (80%)	27
Sporocytophaga: *S. myxococcoides* (72%)	55
Flexibacter: *F. elegans* (33%), *F. polymorphus* (22%)	40, 65
Fruiting gliding	
Myxococcus: *M. coralloides* (51%), *M. falvescens*[b] (70%), *M. fulvus* (45%), *M. macrosporus* (44%), *M. stipitatus* (68%), *M. virescens* (51%), *M. xanthus* (68%)	169
Archangium: *A. gephyra* (18%)	169
Stigmatella: *S. aurantiaca* (58%)	40
Actinomycetes	
Nocardioform	
Oerskovia: *O. turbata* (42%), *O. xanthineolytica* (96%)	21, 124
Saccharomonospora: *S. viridis*	5
Thermopolyspora: T. glauca[a] (73%), *T. (Micropolyspora)*[a] *polyspora* (76%)	5
With Multilocular Sporangia	
Frankia species (55%)	149
Actinoplantes	
Actinoplanes: *A. philippinensis* (51%)	5
Micromonospora: *M. chalcea* (88%), *M. fusca*[b] (53%), *M. globosa* (70%)	5
Streptomycetes and Related Genera	
Streptomyces: *S. afghaniensis* (39%), *S. amakusaensis* (70%), *S. arenae* (47%), *S. aureofaciens* (50%), *S. azureus* (55%), *S. bellus* (44%), *S. bicolor*[b] (58%), *S. caelestis* (68%), *S. canescens* (56%), *S. chartreusis* (45%), *S. chryseus* (61%), *S. cinnabarinus* (61%), *S. coelicolor* (84%), *S. coeruleofuscus* (50%), *S. coeruleorubidus* (56%), *S. coerulescens* (46%), *S. collinus* (44%), *S. coralus*[b] (52%), *S. curacoi* (69%), *S. cyaneus* (58%), *S. echinatus* (66%), *S. erythraeus* (88%), *S. flavovirens* (85%), *S. fumanus* (65%), *S. gardneri* (85%), *S. gelaticus* (79%), *S. glomeroaurantiacus* (65%), *S. gougerotii* (49%), *S. griseorubiginosus* (43%), *S. griseus* (67%), *S. halstedii* (64%), *S. hawaiiensis* (65%), *S. iakyrus* (57%), *S. janthinus* (50%), *S. jumonijiensis*[b] (57%), *S. katrae* (64%), *S. lavendulae* (67%), *S. longisporus* (56%), *S. luteogriseus* (55%), *S. mediterranei* (63%), *S. neyagawaensis* (64%), *S. pullidus*[b] (42%), *S. paradoxus* (64%), *S. peruviensis*[b] (63%), *S. phaeoviridis* (45%), *S. pseudovenezuelae* (56%), *S. resistomycificus* (63%), *S. roseo-luteus*[b] (70%), *S. roseoviolaceus* (36%), *S. rutgersensis* (62%), *S. steffisburgensis*[b] (53%), *S. thermotolerans*[b] (60%), *S. toyocaensis*[b] (37%), *S. venezuelae* (88%), *S. violochromogenes*[b] (47%), *S. violarus* (50%), *S. violatus* (61%), *S. viridochromogenes* (80%), *S. viridis*[b] (64%)	53, 85, 129, 152
Maduromycetes	
Microbispora: *M. amethystogenes* (32%), *M. chromogenes* (46%), *M. diastatica* (46%), *M. parva* (46%), *M. rosea* (59%)	5
Streptosporangium: *S. album* (32%), *S. amethystogenes* (72%), *S. roseum* (69%), *S. viridialbum* (47%), *S. vulgare* (32%)	5
Thermomonospora and Related Genera	
Thermomonospora: *T. curvata* (72%), *T. vividis* (65%)	5
Thermoactinomyces: T. glaucus (63%)	5
Nocardiopsis: *N. alborubidus*[b,f] (50%), *N. albus* subsp. *albus*[f] (47%), *N. albus* subsp. *prasina*[f] (51%), *N. dassonvillei* (53%), *N. listeri*[b,f] (61%)	49
Other genera	
Saccharothrix: *S. coeruleofusca*[g] (58%), *S. flava*[g] (69%), *S. longispora*[g] (61%), *S. mutabilis*[g] (56%), *S. syringae*[g] (74%)	49

[a] Redefined genus or species.
[b] Species which are not listed in *Bergey's Manual of Systematic Bacteriology*.
[c] ω-Cyclohexyl fatty acids.
[d] ω-Cycloheptyl fatty acids.
[e] 12-Methylhexadecanoic acid.
[f] Tuberculostearic acid (6 to 19%).
[g] No tuberculostearic acid.

size and control of the pools of branched-chain amino acids and related intermediates in *E. coli* and *Salmonella typhimurium* have been intensively studied (153). In *E. coli* the pool of α-keto acid intermediates is undetectable, whereas that of branched-chain amino acids ranges from 60 to 300 μm. No similar data are available for bacteria with a branched-chain lipid type. However, in *B. subtilis* a pool size of branched-chain amino acids similar to that of *E. coli* is assumed by judging from the effect on fatty acid composition of varying the concentrations of these amino acids added to the culture medium (68).

The relative activity of the α-keto substrates as primers is another important factor in determining fatty acid pattern. In *B. subtilis* and *B. cereus*, the relative activity is in the order of α-keto-β-methylvalerate ≥ α-ketoisocaproate >> α-ketoisovalerate (76, 113).

Straight-chain fatty acids. Many bacteria listed in Table 3, including the *Bacillus* species, possess a very small proportion of straight-chain fatty acids (0 to 10% of the total fatty acids), the remainder being branched-chain fatty acids. Some species, however, possess straight-chain fatty acids in a considerably higher proportion. The question that arises is that of how so much straight-chain fatty acid could be synthesized even though acetyl-CoA (the primer for the synthesis of these fatty acids) accounts for only a small percentage of the activity of branched-chain primers for branched-chain fatty acid synthetase (83). It is noteworthy that *B. insolitus*, which possesses a high proportion (36% of the total) of straight-chain fatty acids, has a fatty acid synthetase activity as high as 140 times that of *B. subtilis* (82, 83). This indicates that the activity of the condensing enzyme in *B. insolitus* is high in comparison with that of its counterpart in *B. subtilis*. Thus, a large amount of acetyl-ACP would be synthesized from malonyl-CoA by two reactions (Fig. 2) for straight-chain fatty acid synthesis provided that a sufficient supply of malonyl-CoA is maintained in the cells. The direct relationship between the activity of the condensing enzyme and the abundance of straight-chain fatty acids in a bacterium with the branched-chain lipid type remains to be established.

Unsaturated fatty acids. In bacteria with a branched-chain lipid type, anteiso-C_{15} acid has a similar function to that of unsaturated fatty acids in bacteria with a straight-chain lipid type. Thus the former bacteria possess either a small amount of monounsaturated fatty acids or none at all. In fact, most *Bacillus* species have insignificant amounts of unsaturated fatty acids (77). They appear not to require this class of fatty acids for growth, since no mutant of *B. subtilis* requiring unsaturated fatty acids has been reported. This is not true, however, for *E. coli*. A number of *E. coli* mutants which require unsaturated fatty acids have been isolated (136). In support of this general trend, within the genus *Desulfotomaculum*, *D. acetoxidans*, *D. orientis*, and *D. ruminis*, which have a straight-chain lipid type, possess unsaturated fatty acids in a range from 29 to 63% of the total fatty acids, whereas in *D. nigrificans*, which is a branched-chain lipid type, unsaturated fatty acids account for only 6% of the total fatty acids. The same trend is observed in *Clostridium* species (17, 39).

Unsaturated fatty acids are synthesized by two different mechanisms: anaerobic and aerobic (6). An example of the former is the *E. coli* system, which produces *cis*-11-octadecenoic acid (6). Examples of the latter (aerobic) are the *Bacillus* species, which synthesize *cis*-Δ5 and -Δ11 isomers of monounsaturated fatty acids (29, 72). The enzymes involved in the desaturation of fatty acids have recently been studied in detail (43).

Unique fatty acids. Branched-chain fatty acid synthetase can synthesize a wide variety of unusual and/or unnatural fatty acids provided that it is supplied with appropriate primers. For instance, *B. subtilis* produces ω-cyclopropyl, ω-cyclobutyryl, ω-cyclopentanyl, ω-cyclohexanyl, and ω-cycloheptanyl fatty acids if cyclopropyl, cyclobutyryl, cyclopentanyl, cyclohexanyl, and cycloheptanyl carboxylic acids, respectively, are provided to the culture medium (35). It is noteworthy that ω-cyclohexanyl fatty acids occur in *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Curtobacterium pusillum* as the major fatty acids (31, 34, 143), whereas ω-cycloheptanyl fatty acids are the major fatty acids in *Bacillus cycloheptanicus* (32). These unique fatty acids are very useful in bacterial systematics.

Hydroxy fatty acids. 3-Hydroxy straight-chain fatty acids occur in certain gram-negative bacteria, such as *Pseudomonas* and *Serratia* spp., as the major acyl component of lipopolysaccharides in cell walls. Bacteria with the branched-chain lipid system do not usually have these hydroxy fatty acids, but many *Bacteroides*, *Cytophaga*, and *Myxococcus* species and some *Flavobacterium* and *Flexibacter* species are exceptions, having 2- or 3-hydroxy branched-chain fatty acids as major acids (103, 121, 162, 169).

Interestingly, 3-hydroxy-15-methylhexadecanoic acid from lipopolysaccharides of *Bacteroides fragilis* is the only branched-chain hydroxy fatty acid and is in an amide form. The other hydroxy fatty acids are all straight-chain acids with 14, 15, 16, and 17 carbons and are in an ester or ester and amide form (167). The occurrence of branched-chain hydroxy fatty acids in gram-negative bacteria is rather specific and useful in their systematics.

FUNCTION OF BRANCHED-CHAIN FATTY ACIDS

Membrane Components

It has been shown that the appropriate fluidity of membrane lipids provided by the appropriate fatty acid composition at a given growth temperature is a prerequisite for a bacterium. Under conditions where the supply of fatty acids for membrane lipids depends upon the exogenous fatty acids in the medium, the permissible growth temperature for *Acholeplasma laidlawii* B is determined by the fatty acids added to the medium (105).

The fluidity of membrane lipids is related to the average melting point of their respective fatty acid compositions (79). The phase transition temperature (T_m), however, is even more closely related to the fluidity than is the average melting point. Recently, data have become available on the phase transition of chemically synthesized diacylphosphatidylcholine samples. Table 4 lists some of these data. The melting points of a normal acid and an iso-acid with the same number of carbons are similar; however, their phase transition temperatures are significantly different, with the iso-acyl phosphatidylcholine having a T_m ranging from 18 to 28°C below that of the corresponding normal saturated acyl phosphatidylcholine (Table 4) (137). This physicochemical difference among normal, iso-, and anteiso-acids is in accordance with the positional preference in the incorporation of these three types of fatty acids into two positions of membrane phospholipids. For instance, in *B. subtilis*, among C_{15} acids, *n*-C_{15} acid is incorporated mostly into the 1-position of phospholipids; anteiso-C_{15} acid is incorporated exclusively

TABLE 4. Phase transition temperature of diacylphophatidylcholine[a]

Fatty acid	Diacylphophatidylcholine T_m (°C)	Fatty acid melting point (°C)
n-C_{14}	24.0	53.9
n-C_{15}	34.2	52.5
n-C_{16}	41.5	63.1
n-C_{17}	48.8	61.3
n-C_{18}	54.8	69.6
Iso-C_{14}	6.5	53.3
Iso-C_{15}	6.5	51.7
Iso-C_{16}	22.0	62.4
Iso-C_{17}	27.0	60.2
Iso-C_{18}	36.5	69.5
Anteiso-C_{15}	−16.5	23.0
Anteiso-C_{17}	7.6	36.8
ω-Cyclohexyl-C_{17}	18.3	
ω-Cyclohexyl-C_{19}	34.9	

[a] Reproduced from references 96 and 137 with permission.

into the 2-position; and iso-C_{15} acid is in between the two acids, incorporated into both the 1- and 2-positions (74).

Several unsaturated straight-chain fatty acids requiring mutants of *E. coli* have been isolated (136). The growth of these mutants is supported by unsaturated fatty acids (such as oleic and linoleic acids) as well as by branched-chain fatty acids of the iso and anteiso series present in *B. subtilis* (95). All of the above-mentioned fatty acids increase the fluidity of membrane lipids to an acceptable level for growth. In the absence of these fatty acids, the saturated fatty acids normally synthesized by the *E. coli* mutants yield a membrane lipid fluidity level which is too low to support their growth. The properties and structure of cell membranes of the *E. coli* mutants whose growth is supported by branched-chain fatty acids are significantly different from those grown with unsaturated fatty acids (95).

Willecke and Pardee have isolated two mutants of *B. subtilis* that require branched short-chain fatty acids for growth (164). Results of their studies show that growth of the mutants is supported by any one of the following: isobutyric acid, isovaleric acid, or 2-methylbutyric acid. The growth was also supported by ω-alicyclic acids with C_3, C_4, C_5 and C_6 rings (35). However, it was not supported by any of the many other straight and branched short-chain fatty acids tested.

The minimum amount of branched-chain fatty acids needed for growth has been determined by growing *B. subtilis* mutants requiring branched short-chain fatty acids in a medium supplemented with a series of progressively smaller amounts of isobutyric or 2-methylbutyric acid. It is 28% of the total cellular fatty acids; the remainder are straight-chain fatty acids (mainly palmitic acid) (81).

Protonophore-resistant mutants of *B. subtilis* have a higher ratio of iso-C_{15} to anteiso-C_{15} than the parent strain. When the mutants are grown in the culture medium containing palmitoleic acid, its relative proportion in the cultures increases, and the ratio of iso-C_{15} to anteiso-C_{15} is reduced to a level similar to that of the parent strain (89). The composition of membrane fatty acids is altered by varying the growth temperature to maintain the proper membrane fluidity at a given temperature. In bacteria with the straight-chain lipid system, unsaturated fatty acids and short-chain fatty acids increase as the growth temperature is lowered. Four variants of *Bacillus megaterium* grown in the temperature range of 5 to 70°C showed the following changes in the composition of membrane fatty acids with increased growth temperatures: the relative amount of iso-fatty acids increases whereas that of anteiso-fatty acids decreases; and the relative amount of long-chain acids (C_{16} to C_{18}) increases whereas that of short-chain acids (C_{14} to C_{15}) decreases (128). A similar trend has been observed in a Thermus species (126), as well as in *B. stearothermophilus* (120), *B. cereus* (75), *B. caldolyticus*, and *B. caldotenax* (161). A strain of *B. stearothermophilus*, however, adjusts the fluidity of membranes by increasing the proportion of palmitic acid as the growth temperature rises (107).

The composition of membrane fatty acids of host organisms significantly affects the absorption, penetration, and production of viruses. Work with *A. laidlawii* and its lipid-containing virus provides a good example (140). Absorption of the viruses by hosts with homologous membrane lipid acyl-chain composition is poor, whereas absorption by hosts with highly different acyl-chain composition is much greater. This organism possesses only straight-chain fatty acids. It would be interesting to know the effect of branched-chain fatty acids of cell membranes on the absorption of viruses by host bacteria.

It is well established that transport across the membranes of bacterial cells is affected by the physical state of the membranes, and thus by their phase transition temperature (106). The involvement of conformation of membranes due to the structure of acyl chains in transport, however, has not been widely studied. The transport of branched-chain amino acids in *Pseudomonas aeruginosa* (154) and *Streptococcus cremoris* (36) requires phospholipids. The transport in *P. aeruginosa* is dependent on the acyl-chain length of phospholipids (154). Cells with either (+)-anteiso- or (−)-anteiso-acids, like nearly all membrane fatty acids, can be prepared by using *B. subtilis* mutants (81). These two types of cells exhibit stereoselectivity towards L-isoleucine and L-alloisoleucine in their transport (66a).

B. acidocaldarius possesses ω-cyclohexanyl fatty acids. Its mutants, prepared by ethyl methanesulfate treatment, could be grown by supplementation with branched-chain amino acids and cyclohexanecarboxylic acid; they produced branched-chain fatty acids and ω-cyclohexyl fatty acids, respectively, as major cellular fatty acids. Cultures of the mutants grown in the medium supplemented with cyclohexanecarboxylic acid always gave a higher yield of cells, grew at a higher temperature and a lower pH, and had a higher transport activity than those grown with branched-chain amino acids (88).

Activators for Enzymes and Systems

Recently, protein kinase C has been shown to be activated by Ca^{2+} in the presence of phospholipids. 4-Butyl, 7-butyl, and 8-phenyl (but not 8-methyl) derivatives of stearate are equally effective as activators in animal systems as dioleate is (156, 170).

Cholesteryl 14-methylhexadecanoate is known to be required as a cofactor for the incorporation of amino acids into tRNA (147). This ester is a component of animal amino acid-tRNA ligases in changing the conformation of protein synthesis factors and is present in a larger quantity than cholesteryl laurate or cholesteryl palmitate.

The *Cytophaga* and *Flexibacter* group of gliding bacteria produce an unusual sulfonolipid, capnine (2-amino-3-hydroxy-15-methylhexadecane-1-sulfonic acid), as a major lipid; it is shown to be essential for gliding motility (1, 45).

13-Methyltetradecanoic acid, a major fatty acid of these bacteria, is expected to be a precursor of the sulfonolipid.

Protein Modifiers

Fatty acylation of proteins has been found in a wide variety of prokaryotic as well as eukaryotic cells (132, 146). Many proteins are acylated with either myristic or palmitic acid; myristate occurs largely in an amide form, whereas palmitate exists in a thioester form. Most of these studies have been done with cells which have straight-chain fatty acid synthetase. An obvious question is that of which particular acids are substitutions for myristic and palmitic acid in cells having branched-chain fatty acids synthetase. This is virtually unexplored. A study with *Micrococcus luteus* has shown that palmitic acid and other straight-chain fatty acids were enriched in the acylated protein fraction (40% of the total fatty acids) and were mostly in the form of amides (83%), whereas branched-chain C_{15} acids were largely in the form of thioesters (64%) (162). This seems to contradict the general rule mentioned above, since palmitic acid, which is less polar than branched-chain fatty acids, is expected to occur mainly in an amide form. Further studies are required to establish a general rule for cells having branched-chain fatty acids as major acids.

Little is known about the function of proteins acylated by branched-chain fatty acids. The membrane penicillinase of *B. licheniformis* has been shown to contain diglyceride linked to an N-acylated cysteine residue through a thioether linkage (91).

UNUSUAL FATTY ACIDS OF EXTREMOPHILES

Bacteria capable of growing in extreme environments (extremophiles) are required to provide protective measures which are essential in coping with adverse conditions. Cell membranes are an obvious target when subjected to severe treatments.

Low Temperature

Three psychrophilic *Bacillus* species have unusually high proportions, for this genus, of relatively rare unsaturated fatty acids, namely Δ^5-isomers which make up 17, 25, and 28% of the total fatty acids, respectively. Two of the species, in particular, possess very high proportions of straight-chain fatty acids (72, 83). These unsaturated fatty acids are essential for the psychrophiles to adjust the membrane fluidity to the proper level for growth at low temperatures. It should be noted that the phase transition temperature of phosphatidylcholine with di-Δ^5-hexadecenoic acid is significantly higher than that of phosphatidylcholine with di-Δ^9-hexadecenoic acid (a common acid) or di-Δ^{11}-hexadecenoic acid (a *Bacillus* acid) (137). Thus the membrane lipids with the Δ^5-isomers in these *Bacillus* species appear to offer no obvious advantage over the membrane lipids with the Δ^9- or Δ^{11}-isomers for growth at low temperature, but further work is required to determine this point. The presence of a high proportion of straight-chain saturated fatty acids in a *Bacillus* species is advantageous because this class of acids provides a better substrate for desaturase than branched-chain fatty acids (29). A *Pseudomonas* species has been isolated from an Antarctic soil sample. This organism had 90% anteiso-C_{15} acid when it was grown in nutrient-rich medium at 5°C (42). This would be noteworthy although the organism does not possess any unusual fatty acid.

High Temperature and Acidity

Most thermophiles, which grow at high temperatures and at neutral pH (such as *B. stearothermophilus*, *Thermus aquaticus*, and *Flavobacterium thermophilum*), do not possess unusual fatty acids, although the proportions of iso- and anteiso-fatty acids are adjusted for growth at high temperatures (120). Thermophiles which grow under acidic conditions, however, possess unusual fatty acids. Two such organisms, *B. acidocaldarius* and *B. acidoterrestris*, have ω-cyclohexyl fatty acids as major cellular acids, in addition to minor iso- and anteiso-fatty acids (28, 31). Growth under these extreme acidic conditions at high temperature has been used to screen for certain bacteria having uncommon membrane fatty acids and has been successful in yielding an unusual *Bacillus* species, *B. cycloheptanicus*, which contains ω-cycloheptanyl fatty acids as major acids (32, 125).

Alkalinity

Recently, many bacteria capable of growing at a pH as high as 10.5 have been isolated for physiological interest as well as industrial applications. Such bacteria include two *Bacillus* species: *B. firmus* and *B. alcalophilus*. Like the other *Bacillus* species, they possess branched-chain fatty acids as major acids. However, unlike many other species, these contain unusually high proportions of unsaturated fatty acids (20% of the total fatty acids), which are mostly branched-chain fatty acids. In addition they have appreciable amounts of squalene and C_{40} isoprenoids (18). The function of membrane unsaturated fatty acids in the growth of these bacteria under conditions of high alkalinity remains to be discovered, but it may be associated with squalene.

High Salts

Many halophilic bacteria are archaebacteria and possess isoprenoid glycerol ethers as the membrane lipids as opposed to the acyl glycerol esters contained in most bacteria (33). Bacteria capable of growing in oil brines with a high concentration of salts, and in soil with zinc added, include *Curtobacterium pusillum*, which contains cyclohexyl fatty acids as major acids (58, 116).

CONCLUDING REMARKS

It is now clear that bacteria can be divided into three distinct groups on the basis of their membrane lipids. The first group consists of bacteria possessing cell membranes composed of straight-chain acyl esters. Most bacteria are members of this group. The second group has cell membranes composed of branched-chain and alicyclic acyl esters. This includes a significant portion (about 10%) of bacterial species. The third group has cell membranes composed of isoprenoid ethers. This includes a small portion of bacterial species, all of which are archaebacteria (33).

Archaebacteria are uniform with respect to their membrane lipid type and provide an attractive data set of rRNA sequences from which to make inferences about their evolution (47). In contrast, this is not the case for bacteria having branched-chain lipid membranes. I have previously discussed the evolutionary significance of the branched-chain membrane lipid system as an ancient system, which emerged before the appearance of the oxygenic atmosphere on the Earth (77). Evidence to substantiate this idea, however, is yet to come. Nevertheless, the significance of the

branched-chain membrane lipid type in bacterial classification is impressive, particularly so in redefining coryneform bacteria. This trend is expected to continue.

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